2009 Annual Report

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Principal Investigator: John W. Frost

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2367 Science Parkway, Ste. 2
Okemos, MI 48864

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Technical Report

a. Scientific and Technical Objectives

The current microbial synthesis of D-1,2,4-butanetriol using *E. coli* KIT18/pWN7.126B is based on the use of D-xylose as the starting material. Although D-xylose is abundant in hemicellulose, streams of D-xylose sufficiently pure to support microbial growth are not available in the U.S. Because of the current expense of D-xylose, a microbial synthesis of D-1,2,4-butanetriol from D-glucose was targeted for development. The activity of *mdlC*-encoded benzoylformate decarboxylase is essential to improving the yields and concentrations of microbe-synthesized D-1,2,4-butanetriol. Attempt to identify new source of 3-deoxy-D-*glycero*-pentulosonate decarboxylase was carried out. Reaction engineering to improve microbial synthesis of D-1,2,4-butanetriol titer and yield was also examined.

b. Approach

Employing reaction engineering in the current fermentor-controlled microbial synthesis of D-1,2,4-butanetriol from D-xylose, *E. coli* KIT18/pWN7.126B was evaluated under different conditions. Previously, *E. coli* WY9 was created that synthesized 2 g/L of D-xylonic acid in rich LB-xylose medium. Defined minimal salt medium was formulated to enable high-density cultivation of WY9 under fed-batch fermentor-controlled conditions. In parallel with these efforts, microbial synthesis of D-1,2,4-butanetriol from D-glucose using a single *E. coli* microbe was examined. In search for novel keto-acid decarboxylase activity that uses 3-deoxy-D-*glycero*-pentulosonic acid as substrate, an effort combining bioinformatics, codon-optimization and *de novo* gene synthesis was carried out.

c. Concise Accomplishments

Reaction engineering led to a substantial improvement in D-1,2,4-butanetriol titer. *E. coli* KIT18/pWN7.126B synthesized 35 g/L of D-1,2,4-butanetriol from D-xylose under fed-batch fermentor-controlled conditions. *E. coli* WY9/pWY1 synthesized 5.5 g/L of D-xylonic acid from D-glucose using defined minimal medium under fed-batch fermentor-controlled conditions when supplemented with D-ribose. A two-step microbial synthesis of D-1,2,4-butanetriol from D-glucose was formulated using *E. coli* WY9/pWY1 and DH5α/pWN6.186A as biocatalysts. Attempt to synthesize D-1,2,4-butanetriol from D-glucose in a single step using *E. coli* WY9/pWN7.126B was unsuccessful. Novel 3-deoxy-D-*glycero*-pentulosonate decarboxylase activity was identified using codon-optimized *kivD*-encoded 2-ketoisovalerate decarboxylase.

d. Expanded Accomplishments

E. coli WY9/pWY1 was constructed to convert D-glucose into D-xylonic acid. Conversion of D-glucose into D-xylonic acid (Figure 1) required inactivation of D-ribose 5-phosphate isomerase, which is encoded by *rpiA* and *rpiB*, and inactivation of the major transketolase, which is encoded by *tktA*. Leaving *tktB* gene intact in WY9 led to a more stable *E. coli* that remains culturable in minimal salt medium, while retaining the ability to synthesize D-xylonic acid. Nutritional requirement was assessed to culture WY9/pWY1 in minimal salt medium. With the supplementation of 2 g/L D-ribose, *E. coli* WY9/pWY1 was able to synthesize 5.5 g/L D-xylonic acid in minimal salt medium under fed-batch fermentor-controlled conditions.

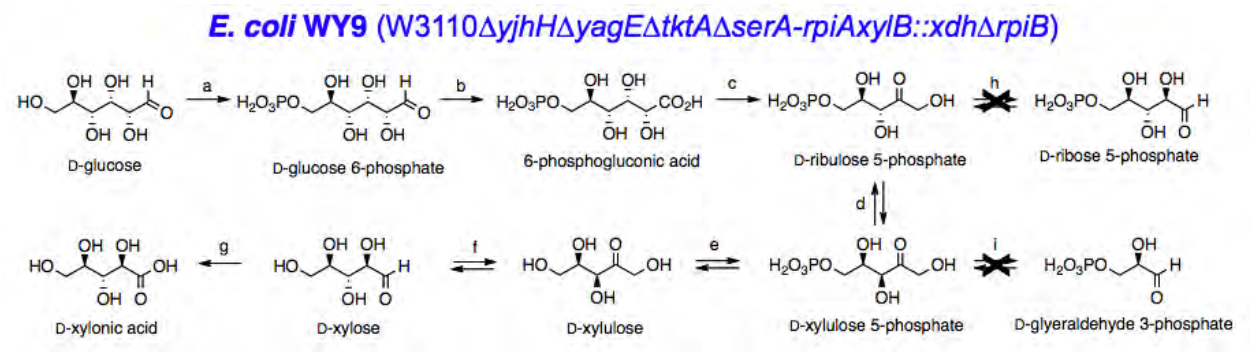

Figure 1. Microbial synthesis of D-xylonic acid from D-glucose. (a) carbohydrate phosphotransferase (*ptsG, crr*); (b) D-glucose 6-phosphate dehydrogenase (*zwf*); (c) 6-phosphogluconate dehydrogenase (*gnd*); (d) D-ribulose phosphate epimerase (*rpe*); (e) phosphatase (*yfbT*); (f) D-xylulose isomerase (*xylA*); (g) *C. crescentus* D-xylose dehydrogenase (*xdh*); (h) D-ribose 5-phosphate isomerase (*rpiA, rpiB*); (i) transketolase (*tktA, tktB*).

A two-step microbial synthesis of D-1,2,4-butanetriol from D-glucose was formulated (Figure 2). *E. coli* WY9/pWY1 synthesized D-xylonic acid from D-glucose. The second *E. coli* biocatalyst DH5α/pWN6.186A converts D-xylonic acid into D-1,2,4-butanetriol. *E. coli* DH5α/pWN6.186A carries a *P. putida mdlC* plasmid insert encoding benzoylformate decarboxylase while relying on native D-xylonate transport along with native D-xylonate dehydratase and dehydrogenase activities. Attempt to synthesize D-1,2,4-butanetriol from D-glucose in a single step using *E. coli* microbe WY9/pWN7.126B was unsuccessful (Figure 3). Plasmid pWN7.126B has a plasmid localized *mdlC* gene cloned under an inducible *tac* promoter. Only D-xylonic acid was isolated from the culture medium of WY9/pWN7.126B.

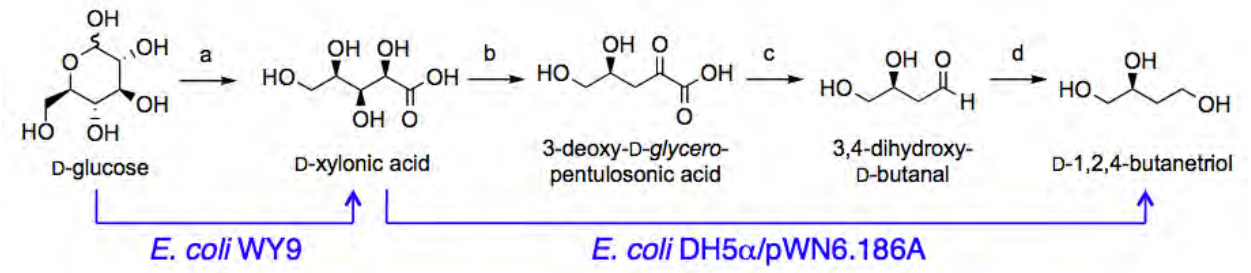

Figure 2. A 2-step microbial synthesis of D-1,2,4-butanetriol from D-glucose. (a) *E. coli* WY9/pWY1; (b) D-xylonate dehydratase (*yjhG, yagF*); (c) 2-keto acid decarboxylase (*P. putida mdlC*); (d) alcohol dehydrogenase.

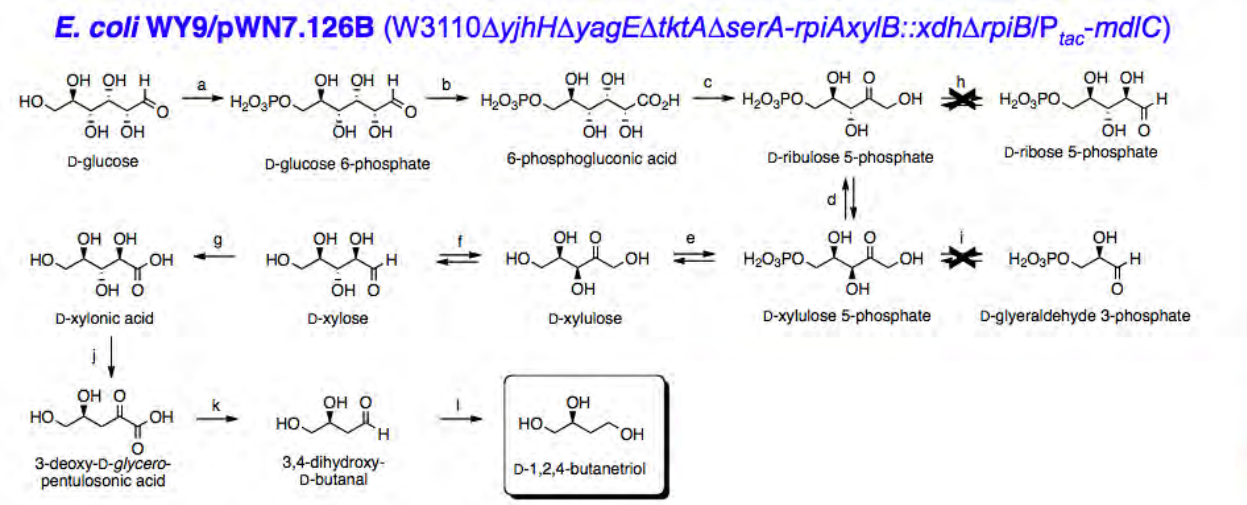

Figure 3. Artificial biosynthetic pathway in *E. coli* WY9/pWN7.126B. (a) carbohydrate phosphotransferase (*ptsG*, *crr*); (b) D-glucose 6-phosphate dehydrogenase (*zwf*); (c) 6-phosphogluconate dehydrogenase (*gnd*); (d) D-ribulose phosphate epimerase (*rpe*); (e) phosphatase (*yfbT*); (f) D-xylulose isomerase (*xylA*); (g) *C. crescentus* D-xylose dehydrogenase (*xdh*); (h) D-ribose 5-phosphate isomerase (*rpiA*, *rpiB*); (i) transketolase (*tktA*, *tktB*); (j) D-xylonate dehydratase (*yjhG*, *yagF*); (k) 2-keto acid decarboxylase (*P. putida mdlC*); (l) alcohol dehydrogenase.

E. coli KIT18/pWN7.126B synthesized 18 g/L D-1,2,4-butanetriol under fed-batch fermentor-controlled conditions. Recently, applying reaction engineering to various fermentation parameters led to a substantial improvement in the product D-1,2,4-butanetriol titer. Production of D-1,2,4-butanetriol under fed-batch fermentor-controlled conditions using KIT18/pWN7.126B was evaluated under different agitation control. As shown in Figure 4, agitation at 700 rpm resulted in the highest product titer at 21 g/L. Agitation at higher rate (900 rpm and 1000 rpm) led to a significant decrease in D-1,2,4-butanetriol production.

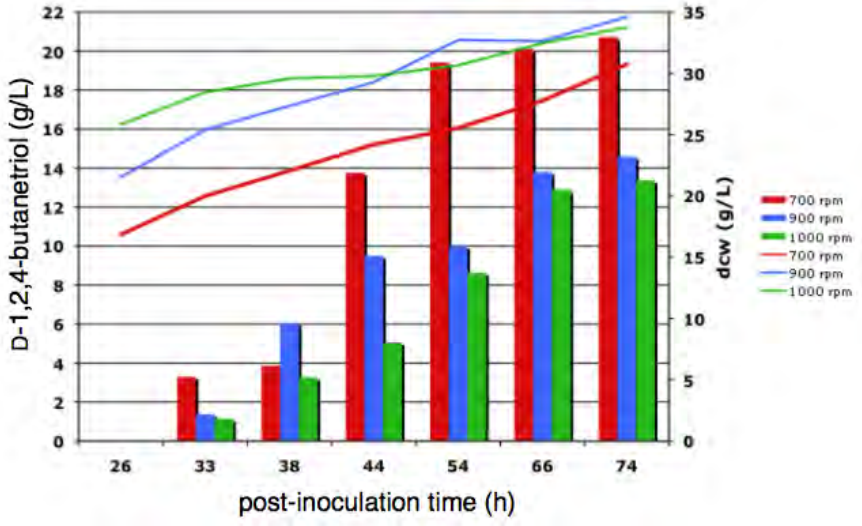

Figure 4. *E. coli* KIT18/pWN7.126B fermentation under different agitation control.

Similar experiments were performed to study different seed culture density, IPTG concentration, dissolved oxygen setting and D-xylose feeding profile (data not shown). Under newly optimized fed-batch fermentor-controlled culturing conditions, E. coli KIT18/pWN7.126B synthesized 35 g/L D-1,2,4-butanetriol in 96 h at a yield of 50% (mol/mol) based on D-xylose consumed (Figure 5).

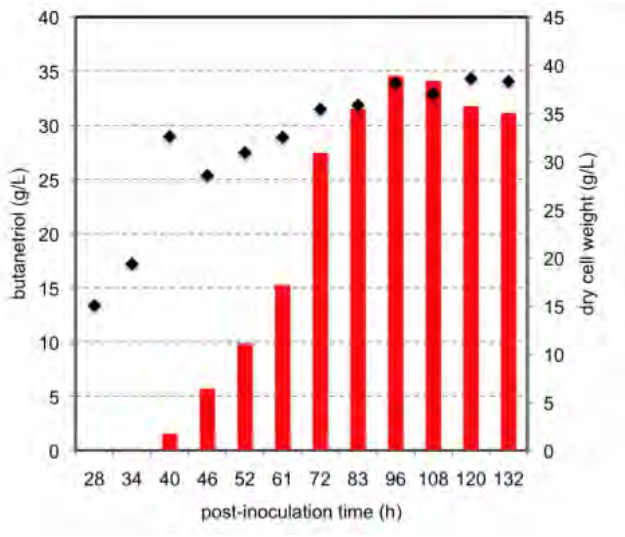

Figure 5. *E. coli* KIT18/pWN7.126B fermentation under optimized culturing conditions.

e. Work Plan

With the successful establishment of a 2-step microbial synthesis of D-1,2,4-butanetriol from D-glucose, attention will now focus on developing a one-microbe, one-step route. Culturing conditions of E. coli WY9/pWN7.126B will be re-evaluated. Prospecting will continue for novel source of decarboxylase activity that uses 3-deoxy-D-*glycero*-pentulosonic acid as substrate.

f. Major Problems/Issues

None.

g. Technology Transfer

h. Foreign Collaborations and Supported Foreign Nationals